Intrinsic viscosity and molecular weight of pectin components*

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ABSTRACT

A variety of fruit and vegetable by-products were extracted to recover and evaluate their pectins. Pectins were studied by high-performance size-exclusion chromatography with differential refractive index (d.r.i.) and differential pressure (d.p.) detection. Computer-aided curve fitting of chromatograms was employed to identify pectic components. For each kind of pectin, the experimental chromatograms from both detectors could be fitted by a linear combination of the same five macromolecular sized species. Combination of data from the d.r.i. and d.p. detectors enabled component and global intrinsic viscosities to be obtained. Universal calibration of the column set with a combination of broad dextran and narrow pullulan standards enabled component and global molecular weights to be obtained. Weight-average intrinsic viscosities, ranged from about 0.75 to 5.9 dL/g, whereas weight-average molecular weights ranged from about 61 to 182 $\times$ 10^3 for the various pectins. Polydispersities of the pectins ranged from about 3 to 11. Analysis by combining intrinsic viscosities with molecular weights and previously determined radii of gyration for the components led to the conclusion that the pectin components were aggregates.

INTRODUCTION

Previously, we found that analysis of various pectins[1,2] by high-performance size-exclusion chromatography (h.p.s.e.c.) with differential refractive index detection (d.r.i.) gave chromatograms that could be reconstructed from a linear combination of five macromolecular sized species. The chromatograms were reconstructed from components by computer curve fitting. In other work, we found that with appropriate column calibration and by connecting differential pressure (d.p.) and d.r.i. detectors in series[3], that intrinsic viscosities (i.v.) and radii of gyration (R_g) could be determined simultaneously by h.p.s.e.c. In this paper, we combine d.r.i., d.p. detection, an alternate form of universal column calibration, and computer-aided curve fitting to obtain molecular weights and intrinsic viscosities of pectin components and the respective

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global averages. These are the same samples for which we previously have determined component weight fractions and radii of gyration[1].

EXPERIMENTAL

Pectins. — All pectins were extracted from fruit and vegetable by-products obtained from food factories in Egypt. Pectin sources investigated were beet pulp, the peels of mangos, oranges, mandarin oranges, grapefruits, pomegranates, and artichokes, the skin of garlic and peas, carrot and colocasia wastes, and garlic foliage. Extraction and carbohydrate analysis have been described elsewhere[1].

H.p.s.e.c. — Chromatography was performed in the usual manner[3]. Pectin solutions were passed through a 0.4–μm nucleopore filter and equilibrated overnight at 35° in capped bottles prior to chromatography. Sample concentrations were adjusted to give a minimum signal-to-noise of 10:1. The injected sample volume was 100 μL. The mobile phase was 0.05M $NaNO_3$ in distilled water passed through a Modulab h.p.l.c. polisher (Continental Water Corp, San Antonio, TX). Solvent was degassed prior to connecting to the system and inline with a model ERC 3120 degasser (Erma Optical Co., Tokyo). The solvent delivery system was a Beckman model 334, (Beckman Instruments Co., Palo Alto, CA). The pumping system was fitted with a Beckman pulse filter and two Waters model M45 pulse dampeners, (Waters Associates, Milford, MA), mounted on a plate and separated by 15 ft of coiled capillary tubing (i.d., 0.01 in). Sample injection was with a Beckman model 210 valve. Three columns were employed in series, a μ-Bondagel E-High, E-1000, Waters Assoc. (300 × 3.9 mm) and a Synchropak GPC-100 (250 × 4.6 mm) (Synchrom, Inc., Linden, IN). The homemade viscosity detector was similar to that described by Malihi *et al.*[4]. The viscosity detector consisted of a 2-ft length of capillary tubing connected with low-volume stainless steel tees to a model P-7 differential tranducer (Celesco, Canoga Park, PA). The pressure transducer was rated to give a full scale (f.s.) electrical output of 10 V at 25 p.s.i. The value of the capillary tubing i.d. was 0.0124 in. Differential refractive index (d.r.i.) was measured with a model ERC 7810 monitor. Chromatography columns and the capillary tubing of the transducer were thermostatted in a temperature-controlled water bath at 35 ± 0.003°, and the cell of the d.r.i. monitor was also thermostatted at 35°. Measurement of flow rate and data acquisition have been previously described by Fishman *et al.*[5] All samples were chromatographed at a nominal flow rate of 0.5 mL/min.

Curve fitting. — Partially resolved, overlapped components of the d.r.i. and d.p. detector chromatograms were determined with the aid of ABACUS, version D.2, a curve-fitting program[2]. ABACUS is a user-interactive, command-driven program which fits chromatograms to a series of up to eight peaks. Gaussian, exponentially modified Gaussian, Lorenzian or any combination of these peak shapes can be chosen. ABACUS determines the position and area of each peak relative to the experimental chromatogram by minimizing the sum of the squares of the residuals between the heights at each point of the calculated curve as compared to the experimental curve. In addition to the experimental envelope and the component peaks, the calculated enve-

lope, when it differs from experimental, is shown on the chromatogram. The least-squares minimization is accomplished by a non-linear approach utilizing the Gauss–Newton iteration technique[6]. The number of component peaks is estimated prior to the Gauss–Newton iteration. Three parameters can be iterated for each component peak; location of maximum, height at maximum, and width. We have found experimentally, after investigating a large variety of pectins over their full size range, that a linear combination of usually five but occasionally three or four Gaussian peaks consistently gave the best fits. In its most general form ABACUS does not give a unique solution to the best fit; therefore, certain safeguards were observed. Those parameters judged to be the best were those which (a) gave lowest values for the sum of the least squares residuals between calculated and experimental curves for d.r.i. and d.p. detectors, (b) gave maximum overlap between envelopes of calculated and experimental chromatograms, and (c) required the minimum number of peaks. It was found that parameters determined for d.r.i. chromatograms consistently gave the best fits for the corresponding d.p. chromatograms. After correcting for the dead volume between detectors, only peak heights have to be iterated because of the different sensitivities of the two detectors to the various pectin components. Values of 0.274 and 0.116 mL were used for the quarter bandwith at half height for the macromolecular sized components and those in the low molecular weight tail, respectively. Determination of these values has been previously described[2].

The dead volume between d.r.i. and d.p. detectors was measured as 125 ± 1 μL by matching the front sides of chromatograms from a narrow P-50 pullulan standard. The point-by-point digitized data from both detectors was normalized (divided) by their respective chromatogram areas. Each set of points was entered as a two-dimensional array (normalized height against volume) in a Lotus 1-2-3 spreadsheet (Release 2.01, Lotus Development Corp., Cambridge, MA) and plotted together using the graphing software of the spreadsheet. Various estimates of the dead volume were subtracted point-by-point from the volumes of the d.r.i. trace until the front side of d.r.i. chromatogram was superimposed on the front side of the d.p. chromatogram.

Column calibration. — Previously, we have obtained congruent calibration curves by plotting log R_g against column partition coefficient, K_{av} for a series of narrow molecular weight distribution (m.w.d.) pullulan and broad m.w.d., dextran standards (*i.e.*, dextrans with polydispersities ranging from 1.39 to 2.91)[5]. Such a calibration curve was used to obtain radii of gyration for pectin samples[1–3,5]. The more usual form of "universal calibration"[7,8] involves plotting log $[\eta]$ M against K_{av}, where M is the molecular weight of the macromolecule and $[\eta]$ is the i.v. In Fig. 1 is a congruent plot for narrow m.w.d. pullulan and broad m.w.d. dextran standards, obtained by plotting log $[\eta]$ M against K_{av}. We found that plotting peak positions for both pullulans and dextrans gave points which were not congruent when the weight average molecular weight of dextran exceeded 110×10^3. The plot became congruent only when the K_{av} was chosen to correspond with the point on the dextran chromatograms where M_w elutes. The method of matching K_{av}'s with M_w's for dextrans and of data reduction, has been described elsewhere[5]. Unlike the dextrans, in the case of chromatograms for the narrow

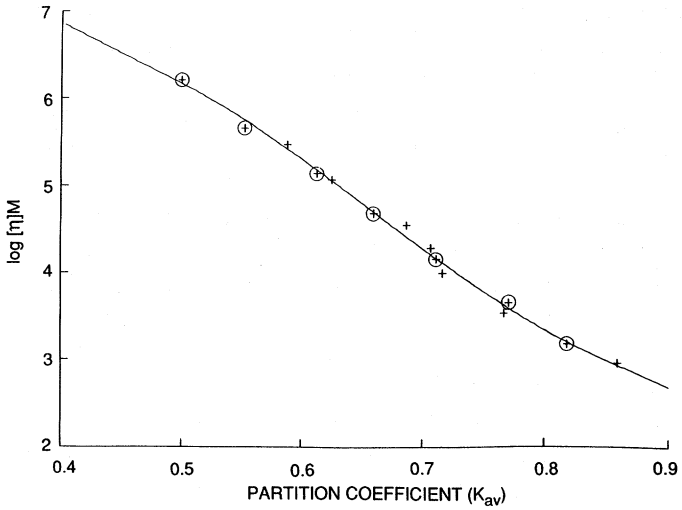


Fig. 1. Calibration curve. Standards in order of elution. In legend: P = pullulan; T = dextran. P800, 3.52 mg/mL; P400, 5.52 mg/mL; T500 16.9 mg/mL; P200 9.24 mg/mL; T250, 16.7 mg/mL; P100 13.9 mg/mL; T110 17.4 mg/mL; T70 20.4 mg/mL; P50 20.8 mg/mL; T40 23.4 mg/mL; T20 23.1 mg/mL; P20 20.9 mg/mL; P10 20.0 mg/mL; T10 24.8 mg/mL. Mobile phase, 0.05M NaCl; nominal flow rate 0.5 mL/min; injection volume, 100 μL; detection, differential refractive index. On graph: $\oplus$ pullulan, + dextran.

pullulan standards, molecular weight could be associated with the K_{av} of the peak position. By assuming that the calibration curve in Fig. 1 is valid for pectins, its molecular weight can be obtained by measuring i.v. as a function of K_{av}.

Calculation of intrinsic, percentage specific viscosity, weight fraction, and global averages. — Previously[1,2], we have shown that the concentration or weight fraction of each subunit can be calculated from their respective refractive index areas. It is readily shown that the i.v. of the subunit can be calculated from the respective component areas of the differential pressure (d.p.) and refractive index (d.r.i.) traces according to Eqs. *1* and *2*.

$$[\eta] = \frac{(2\pi)^{\frac{1}{2}} \times \sigma_{Pi} \times h_{Pi}}{W \times w_i \times \Delta P_0} \qquad (1)$$

$$w_i = \frac{h_{RIi}}{\Sigma h_{RIi}} \qquad (2)$$

Where: σ_{Pi} is the quarter width at half height of the ith component peak from the differential pressure chromatogram; h_{Pi} is the height at peak maximum of the ith component peak from the differential pressure chromatogram; w_i is the weight fraction of the ith component; h_{RIi} is the height at peak maximum of the ith component peak from the refractive index chromatogram; $\triangle P_o$ is the differential pressure produced by the mobile phase; W is the weight of injected sample.

Each component's percentage of the specific volume ($\%\eta_{spi}$) was calculated according to Eq. *3*.

$$\%\,\eta_{spi} = \left((2\pi)^{\frac{1}{2}} \times \sigma_{Pi} \times h_{Pi} \times \frac{\Delta P_0}{\int \Delta P_s - \Delta P_0}\right) 100 \tag{3}$$

Where the integral in the denominator of Eq. *3* is the area from the d.p. chromatogram of the injected pectin sample[3].

Number-, weight-, and Z-average global properties were calculated according to Eqs. *4–6*, respectively.

$$x_n = \frac{1}{\Sigma \frac{X_i}{w_i}} \tag{4}$$

$$X_w = \Sigma w_i X_i \tag{5}$$

$$X_z = \frac{\Sigma w_i X_i^2}{\Sigma w_i X_i} \tag{6}$$

Here X represents molecular weight or intrinsic viscosity. In a previous paper[1], global average radii of gyration were calculated by replacing X with R_g in Eqs. *4–6*.

RESULTS AND DISCUSSION

Figs. 2–4 contain the respective chromatograms for pomegranate, carrot, and garlic skin pectins obtained with d.r.i. and d.p. detectors. These chromatograms are representative of the pectins investigated. With the aid of on-line d.r.i. and d.p. detection, it is possible to continuously determine the intrinsic viscosity of a polymer as it is eluted from the column[4]. Computer-aided curve fitting to obtain mathematically resolved components, also serves as a method of obtaining the intrinsic viscosity as a function of elution volume, because the smoothed envelope of the experimental chromatogram is reproduced as well (see Figs. 2b–4b). In Figs. 5a–5c, the superimposed, smoothed i.v., d.r.i., and d.p. curves are plotted as a function of partition coefficient. At the front and tail ends of the chromatograms, i.v. almost parallels the x-axis. As indicated by the K_{av} values at the front and tail ends of the chromatograms, the sample of pomegranate pectin elutes well within the fractionating range of the column set. In Fig. 5b, i.v. superimposed upon the mathematically resolved components is plotted against K_{av} for the d.r.i. detector, whereas 5c contains comparable data for the viscosity detector. The data of Figs. 5b and 5c show that i.v. is initially constant with elution because pure component 1 elutes. As component 2 starts to elute along with component 1, the overall i.v. decreases because the i.v. of component 2 is less than that of 1 (Table I).

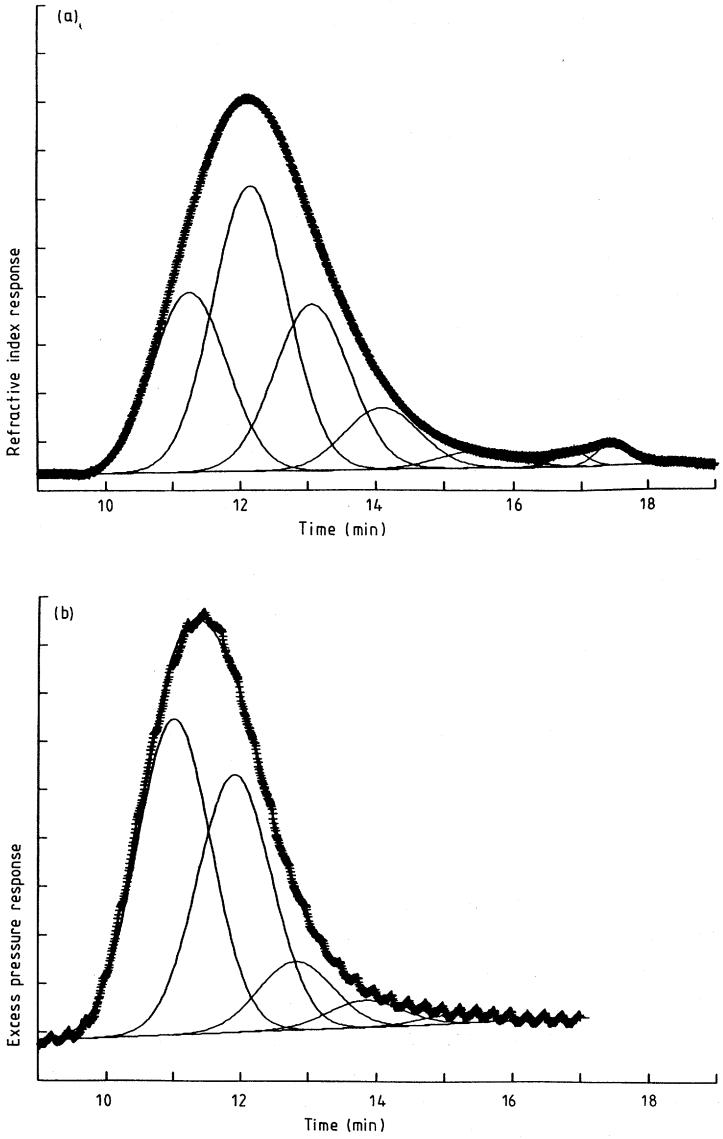


Fig. 2. Chromatograms of pomegranate pectin. Mobile phase, 0.05M NaCl; nominal flow rate, 0.5 mL/min; injection volume, 100 μL; injected concentration 2.64 mg/mL. Thick line, experimental; thin line, calculated; peak components referred to in the text by number are 1–5, left to right. (a) Detector, differential refractive index; (b) detector, differential pressure.

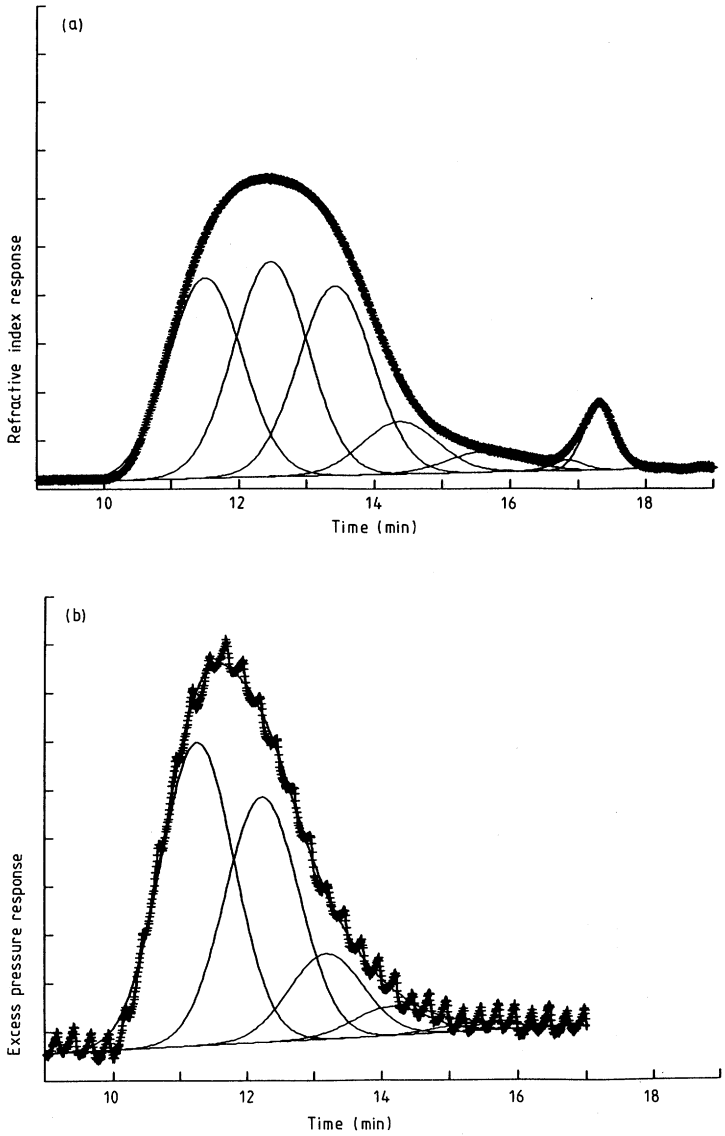


Fig. 3. Chromatograms of carrot pectin; injected concentration 2.60 mg/mL. For all other conditions see Fig. 2.

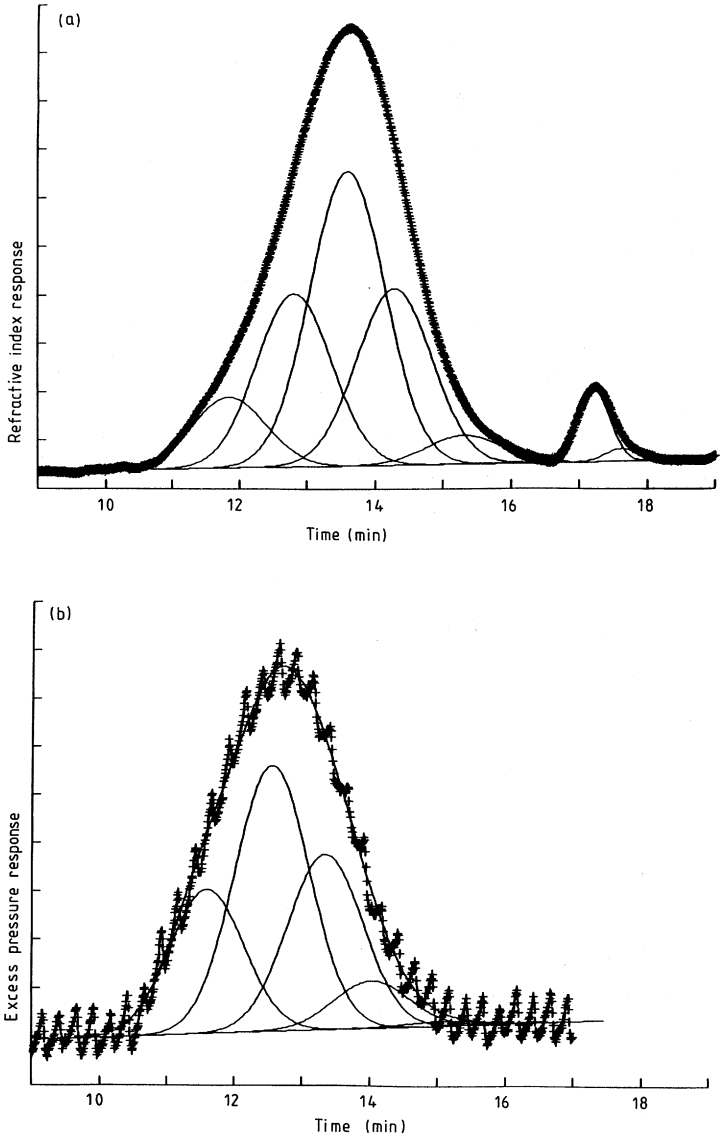


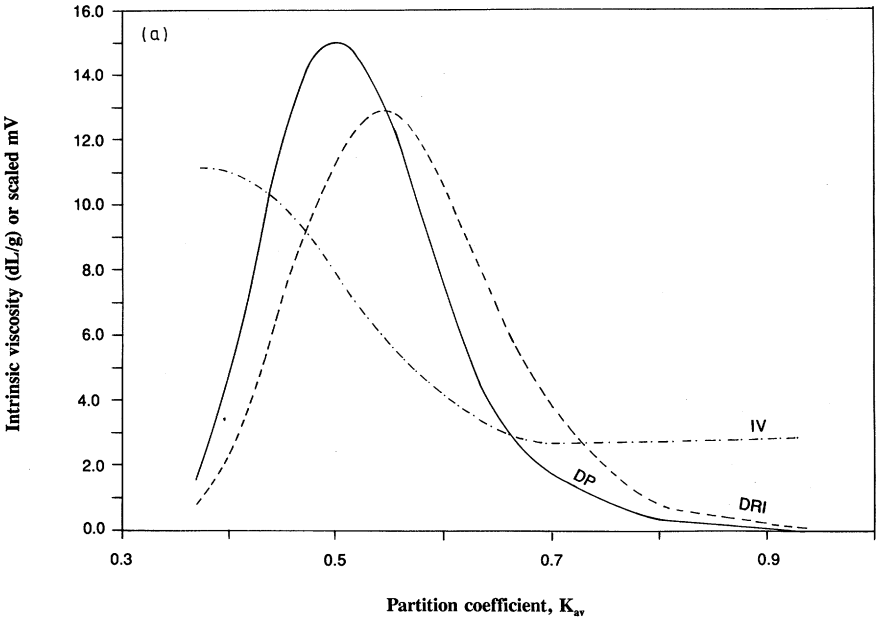
Fig. 4. Chromatograms of garlic skin pectin; injected concentration 2.58 mg/mL. For all other conditions see Fig. 2.

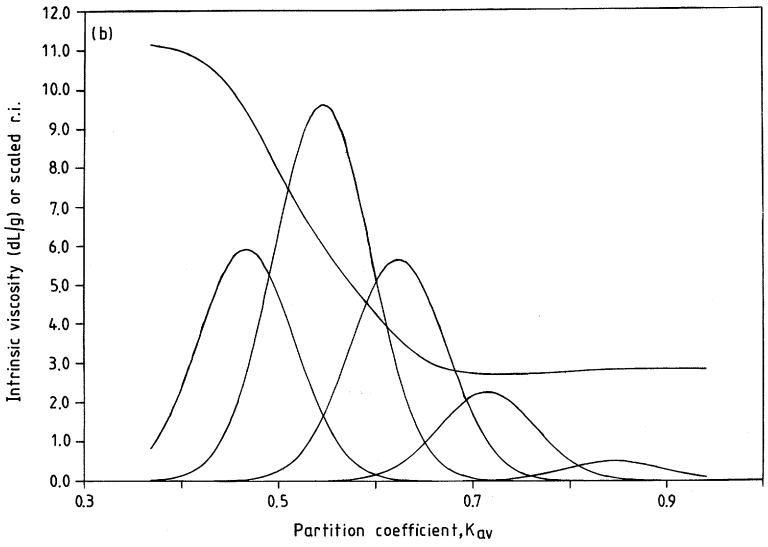
TABLE I

Intrinsic viscosity[a] of pectin components

Sample	*Component Number*				
	1	*2*	*3*	*4*	*5*
Pomegranate	11.0 ± 0.2	5.5 ± 0.3	2.52 ± 0.11	2.73 ± 0.04	2.95 ± 0.60
Carrot	3.6 ± 0.1	2.7 ± 0.1	1.07 ± 0.05	0.99 ± 0.25	1.06 ± 0.49
Beet	4.4 ± 0.1	2.7 ± 0.1	0.94 ± 0.04	0.90 ± 0.23	1.68 ± 0.24
Orange	4.0 ± 0.1	2.9 ± 0.1	0.94 ± 0.04	0.89 ± 0.16	0.82 ± 0.09
Artichoke	5.9 ± 0.2	2.9 ± 0.1	0.95 ± 0.08	0.54 ± 0.12	0.54 ± 0.07
Colocasia	4.2 ± 0.1	2.6 ± 0.2	0.96 ± 0.06	0.46 ± 0.02	1.51 ± 0.22
Mandarin orange	7.5 ± 0.1	4.0 ± 0.2	1.41 ± 0.03	1.64 ± 0.26	1.80 ± 0.30
Mango	4.0 ± 0.1	2.4 ± 0.1	0.86 ± 0.12	0.60 ± 0.19	1.09 ± 0.42
Grapefruit	3.3 ± 0.2	2.3 ± 0.1	0.78 ± 0.06	0.36 ± 0.14	0.74 ± 0.36
Pea skin	4.3 ± 0.1	2.0 ± 0.1	0.67 ± 0.01	0.38 ± 0.08	0.22 ± 0.12
Garlic skin	2.5 ± 0.2	1.7 ± 0.1	0.65 ± 0.03	0.27 ± 0.03	0.38 ± 0.42
Garlic foliage	2.0 ± 0.1	1.4 ± 0.2	0.48 ± 0.07	0.16 ± 0.11	0.54 ± 0.52
Average	4.7 ± 2.7	2.8 ± 1.1	1.02 ± 0.52	0.83 ± 0.72	1.10 ± 0.80

[a] (dL/g)


(a)
Intrinsic viscosity (dL/g) or scaled mV
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0.3
0.5
0.7
0.9
IV
DP
DRI
Partition coefficient, K_{av}


(b)
Intrinsic viscosity (dL/g) or scaled r.i.
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0.3
0.5
0.7
0.9
Partition coefficient, K_{av}

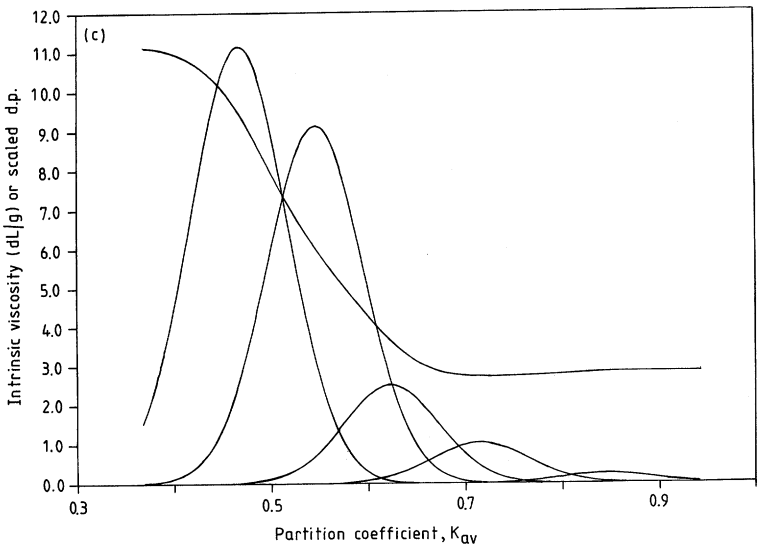


Fig. 5. Pomegranate pectin. (a) Superimposed curves of intrinsic viscosity (i.v.), differential refractive index (d.r.i.), and differential pressure (d.p.) as a function of K_{av}. Calculated from reconstructed chromatograms shown in Figs. 2a and 2b. (b) Intrinsic viscosity superimposed upon fitted components from d.r.i. (c) Intrinsic viscosity superimposed upon fitted components from d.p.

Further decreases in the overall i.v. occur with the sequential elutions of components 2 and 3, because 3 has an i.v. which is less than 2. Since components 3, 4, and 5 have comparable values of i.v., the overall i.v. tends to parallel the x-axis during the elution of these components. Table I contains the intrinsic viscosities of components from the other sources of pectin which were investigated.

Table II contains the percentage of total specific viscosity ($\%\eta_{spi}$) contributed by each component of the pectin sample as calculated from Eq. *3*. On average, about 75% of the sample viscosity is contributed by components 1 and 2, about 94% by the three largest components. Thus relative errors in viscosity tend to be greater for the two smallest components than for the three larger ones. Furthermore, there tends to be greater relative erors in number-average intrinsic viscosities as compared to weight- or Z-averages (Table III).

According to Eq. *7*, which is a modified form of the Einstein viscosity equation[9], the product of intrinsic viscosity and M are proportional to the mean square radius of gyration raised to the 3/2 power.

$$[\eta]M = A(R_g^2)^{\frac{3}{2}} \tag{7}$$

For any particular polymer-solvent system held at constant temperature, A is a constant. Thus, the product of the intrinsic viscosity and M must remain constant if R_g were to remain constant. At constant R_g, a change in i.v. must result in an opposite change in

TABLE II

Percentage specific viscosity of pectin components

Sample	*Component Number*				
	1	*2*	*3*	*4*	*5*
Pomegranate	47.9 ± 2.5	37.3 ± 1.1	9.8 ± 0.2	3.9 ± 0.4	1.0 ± 0.3
Carrot	43.5 ± 1.7	37.6 ± 1.2	13.8 ± 1.4	3.9 ± 0.5	1.3 ± 0.7
Beet	40.1 ± 1.1	41.3 ± 0.4	13.0 ± 1.0	4.2 ± 0.4	1.4 ± 0.3
Orange	33.5 ± 1.6	44.7 ± 0.6	16.2 ± 0.5	4.5 ± 0.7	1.1 ± 0.1
Artichoke	37.6 ± 0.6	42.5 ± 1.7	16.1 ± 0.8	3.1 ± 0.7	0.6 ± 0.2
Colocasia	28.3 ± 2.7	39.2 ± 1.5	22.8 ± 1.3	7.6 ± 0.7	2.1 ± 0.4
Mandarin orange	36.4 ± 0.6	41.6 ± 0.9	15.1 ± 0.5	5.1 ± 0.8	1.8 ± 0.2
Mango	29.8 ± 2.2	43.6 ± 1.1	20.4 ± 3.4	4.8 ± 1.0	1.3 ± 0.7
Grapefruit	26.9 ± 2.6	43.4 ± 1.4	24.3 ± 2.8	4.0 ± 1.6	1.4 ± 0.9
Pea skin	37.5 ± 0.9	43.1 ± 0.3	16.1 ± 0.9	3.1 ± 0.1	0.5 ± 0.4
Garlic skin	23.5 ± 0.9	40.8 ± 0.8	28.4 ± 1.2	6.2 ± 1.2	1.1 ± 1.0
Garlic foliage	21.7 ± 2.1	42.5 ± 3.6	29.0 ± 3.0	5.0 ± 4.3	0.5 ± 0.3
Average	33.9 ± 8.1	41.5 ± 2.4	18.8 ± 6.2	4.6 ± 1.3	1.2 ± 0.5

molecular weight (*e.g.*, an increase in i.v. would require a decrease in molecular weight). Previously Fishman *et al.*[1] found that the respective components 1, 2, and 3 of carrot, beet, orange, artichoke, colocasia, mandarin orange, and mango pectins had similar values of R_g. The same was true for the component 1 R_g of grapefruit, pea, and garlic skin.

Nevertheless, the molecular weight order of component 1 was carrot > beet ~ orange ~ colocasia > mango > mandarin orange (Table IV), whereas the i.v.'s were in reverse order. Furthermore, component 1 molecular weights were in the order, garlic

TABLE III

Global average intrinsic vciscosities[a]

Sample	$[\eta]_n$[b]	$[\eta]_w$[c]	$[\eta]_z$[d]
Pomegranate	4.39 ± 0.07	5.92 ± 0.05	7.70 ± 0.13
Carrot	1.66 ± 0.11	2.25 ± 0.04	2.77 ± 0.02
Beet	1.54 ± 0.08	2.30 ± 0.05	3.09 ± 0.03
Orange	1.43 ± 0.10	2.10 ± 0.06	2.82 ± 0.01
Artichoke	1.22 ± 0.14	2.25 ± 0.09	3.67 ± 0.09
Colocasia	0.93 ± 0.02	1.57 ± 0.03	2.48 ± 0.05
Mandarin orange	2.26 ± 0.09	3.34 ± 0.04	4.74 ± 0.09
Mango	1.09 ± 0.06	1.69 ± 0.02	2.45 ± 0.04
Grapefruit	0.82 ± 0.14	1.42 ± 0.02	2.13 ± 0.11
Pea skin	0.51 ± 0.34	1.55 ± 0.07	2.58 ± 0.02
Garlic skin	0.55 ± 0.04	0.98 ± 0.03	1.50 ± 0.02
Garlic foliage	0.37 ± 0.12	0.75 ± 0.03	1.18 ± 0.04

[a] dL/g. [b] Number average. [c] Weight average. [d] Z-average.

TABLE IV

Molecular weight of pectin components ($\times 10^3$)

Sample	*Component Number*				
	1	*2*	*3*	*4*	*5*
Pomegranate	214 ± 6	106 ± 8	36.8 ± 5.6	3.7 ± 0.9	0.30 ± 0.06
Carrot	475 ± 21	125 ± 11	41.7 ± 4.0	6.8 ± 2.3	1.06 ± 0.49
Beet	383 ± 33	120 ± 12	59.8 ± 8.6	9.7 ± 3.8	0.50 ± 0.08
Orange	404 ± 8	95 ± 6	47.5 ± 5.3	7.0 ± 2.3	0.80 ± 0.12
Artichoke	263 ± 14	109 ± 3	56.0 ± 5.5	16.5 ± 3.3	1.71 ± 0.29
Colocasia	356 ± 35	120 ± 6	49.9 ± 0.7	16.9 ± 0.9	0.72 ± 0.13
Mandarin orange	184 ± 2	62 ± 1	28.4 ± 2.2	3.1 ± 0.7	0.40 ± 0.09
Mango	342 ± 10	111 ± 4	52.7 ± 5.2	12.9 ± 6.1	0.92 ± 0.41
Grape fruit	362 ± 33	96 ± 7	53.6 ± 1.7	22.8 ± 1.7	1.37 ± 0.61
Pea skin	237 ± 5	103 ± 5	51.0 ± 7.7	12.9 ± 7.7	3.95 ± 2.04
Garlic skin	444 ± 22	111 ± 10	51.8 ± 1.7	25.8 ± 3.1	6.40 ± 4.69
Garlic foliage	397 ± 1	88 ± 7	38.6 ± 1.9	29.3 ± 15.3	2.20 ± 2.32
Average	338 ± 93	104 ± 18	47.3 ± 9.8	13.9 ± 9.2	1.69 ± 2.14

skin > grapefruit > pea skin whereas the i.v.'s were in reverse order. Comparison revealed that the molecular weights of components 2 and 3 of mandarin orange were lower than those of carrot, beet, orange, artichoke, colocasia, and mango, whereas the i.v.'s were higher. These results are evidence that pectin components with similar values of R_g and different values of i.v., must be aggregated to different extents. Furthermore, these results are in agreement with previous studies in this laboratory that citrus pectins[2,3,10,11] and tomato pectins[2] are aggregated.

Table V contains number, weight- and Z-average molecular weights of the various pectins calculated from equations 4–6, where w_i and X_i refer to the weight

TABLE V

Global average molecular weights ($\times 10^3$)

Sample	M_n[a]	M_w[b]	M_z[c]	M_w/M_n
Pomegranate	10 ± 2	107 ± 5	156 ± 3	11
Carrot	18 ± 5	182 ± 3	369 ± 9	10
Beet	17 ± 2	141 ± 10	259 ± 19	8
Orange	16 ± 2	120 ± 2	271 ± 10	8
Artichoke	31 ± 4	96 ± 4	155 ± 6	3
Colocasia	18 ± 2	89 ± 2	199 ± 17	5
Mandarin orange	7 ± 1	61 ± 1	115 ± 1	9
Mango	22 ± 6	101 ± 5	196 ± 3	5
Grapefruit	27 ± 10	94 ± 1	200 ± 18	4
Pea skin	39 ± 2	88 ± 1	138 ± 5	3
Garlic skin	37 ± 10	95 ± 3	237 ± 21	3
Garlic foliage	22 ± 9	76 ± 6	205 ± 2	3

[a] Number average. [b] Weight average. [c] Z-Average

fractions and molecular weights of the pectin components respectively. These results reveal that the five-component model is consistent with moderate diversity in the molecular weight distributions of pectins from various sources. Polydispersity, (M_w/M_n), ranges from moderately to extremely broad (*i.e.*, 3–11). Thus, as in the case of pomegranate pectin, it is possible for M_n to be relatively low, 10×10^3 and for M_w to be moderately high, 107×10^3.

Conclusions. — A remarkably simple, empirically derived model enabled us to reconstruct the chromatograms obtained almost simultaneously from a d.p. and a d.r.i. detector connected in series. Furthermore, these chromatograms were obtained from pectins which were derived from a variety of sources. Moreover, the concept that pectin is composed of a linear combination of five macromolecular sized aggregates provides a physico-chemical basis for explaining some of the puzzling and sometimes seemingly contradictory properties of pectin. Finally, the occurrence of these five components in pectins from a variety of sources may indicate that pectins have a quaternary and possibly a sub-unit structure held together by a combination of covalent bonds and non-covalent interactions.

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